IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

Xing SU et al.

Serial No. 09/373,984 Group Art Unit: 1656

Filed: 16 August 1999 Examiner: J. Tung

For: SINGLE-PHASE AMPLIFICATION OF NUCLEIC ACIDS

AMENDMENT IN RESPONSE TO NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES

Commissioner for Patents
Washington, D.C. 20231

Sir:

In response to the Office Action dated 5 October 2000, enclosing the Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures, please amend the above-identified application as follows:

IN THE SPECIFICATION

Please replace the designated paragraphs as follows:

Page 5, lines 6-21 (Amended)

Figure 1 depicts an overview of a preferred embodiment of the single-phase amplification methods of the present invention. PolyA+ or total RNA is annealed with the single-stranded oligo dT-tailed promoter primer, T_7T_{20} (ggC Cag TgA ATT gTA ATA CgA CTC ACT ATA ggg Agg Cgg $(T)_{20}$ (SEQ ID NO. 1)), creating a primer-template mixture. First strand cDNA synthesis is accomplished by combining the first strand cDNA reagent mix (Superscript II, buffer, DTT, and dNTPs) with the primer-template mixture and incubating at the appropriate time and temperature. Second strand cDNA synthesis is then performed by mixing the first strand cDNA reaction with second strand reagent mix, containing secondary cDNA mix (depc-H_20, Tris-HCl (pH7.0), $MgCl_2$, $(NH_4)SO_4$, beta-NAD^+, and dNTPs) and cDNA enzyme mix (Amplitaq DNA polymerase, *E. coli* ligase, *E. coli* RNase H, and *E. coli* DNA polymerase I)), followed by incubation at the appropriate times and temperatures. The

resulting double-stranded (ds) cDNA contains a functional T7 RNA polymerase promoter, which is utilized for transcription. Finally, in vitro transcription is performed by combining the (ds) cDNA with IVT reagent (NTP, buffer, T7 RNA polymerase), yielding amplified, antisense RNA.

Page 13, lines 11-20 (Amended)

Step 1: Primer-template annealing. The HPLC purified primer may be obtained from a -20°C storage stock, prepared in a 100 μM solution with TE (10 mM Tris-HCl (pH 8.0) and 1 mM EDTA (pH 8.0)) and diluted 1:1 with glycerol (for a final concentration of 50 μM in 50% glycerol and 50% TE). Where the desired nucleic acid sample is poly(A)+ RNA, a T_7T_{20} primer (ggc cag tga att gta ata cga ctc act ata ggg agg cgg $(T)_{20}$ (SEQ ID NO. 1)) (Operon Technologies, Inc., Alameda, California) for example, may be used. In such case, an RNA sample (10 to 100 ng mRNA or 1-2 ug total RNA suspended in 2.5 μl or less) can be mixed with 0.5 μl primer to give a final volume of 3 μl. The mixture can be incubated at 70°C for 5-10 minutes, then cooled to 4°C.

Please refer to the attachment for the marked up version of the amended paragraphs, pursuant to revised 37 CFR 1.121(b).

Prior to the Claims, which begin on page 23, please insert the attached Sequence Listing.